

# Infectious Agents and Cancer

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Oral presentation

## Development and application of a real time PCR assay for detection and quantification of merkel cell virus (MCV) in archived formalin fixed paraffin embedded tissue samples of merkel cell carcinoma and other cancers

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### Background

Merkel cell carcinoma (MCC) of the skin is a highly malignant primary cutaneous neuroendocrine malignancy. Older individuals, patients undergoing immunosuppressive therapy for organ transplant, and patients with HIV infection who are immunosuppressed are at a higher risk for developing MCC. MCC may share etiologic influences with other malignancies, and increased risks for other cancers in MCC patients have been reported. Feng et al recently identified a new human polyoma virus, Merkel Cell Virus (MCV), in MCC. The discovery of MCV raises important questions. Is MCV obligatory for MCC? The absence of the viral genome in 20 percent of the 10 MCC cases in the index study suggests that some MCC may not be associated with MCV. What is the natural reservoir for this virus? Feng et al demonstrated low levels of detection in skin samples from non-disease controls. How prevalent is an MCV latent or active infection? Can detection be carried out in archived formalin fixed paraffin embedded (FFPE) tissue samples of MCC to clarify the association between MCV and MCC? Until such time as tools for serology are available polymerase chain reaction (PCR) based detection methods, particularly those that can effectively be applied to small and FFPE samples, will be valuable.

### Methods

A real time PCR assay for MCV was developed using a known source of fresh, frozen MCC with confirmed integrated MCV kindly provided by Dr. Patrick Moore, University of Pittsburgh. The assay readily and reproducibly amplified MCV DNA. To test the assay in FFPE tissues, we assembled a 25-case series of primary and metastatic MCC archived from 1994 to 2008. A tissue microarray (TMA) was constructed to confirm the MCC diagnosis by a battery of immunohistochemistry tests. These 25 FFPE samples included MCC from 22 individual patients some with and some without known immunodeficiency. Duplicate diagnostic blocks were available from nine patients. The PCR samples were coded off site so the identity and inter-relationships of the samples were unavailable to persons performing the PCR assay. In addition to reagent controls, we included DNA from 12 colon carcinomas and 20 breast carcinomas.

### Results

The MCV real-time PCR assay had excellent linearity with an input of genomic DNA in the range of 10 ng to 0.1 ng corresponding to 1000 to 10 cells. With 40 cycles of amplification, DNA from peripheral blood lymphocytes (PBL) used as a negative control reproducibly scored negative for amplification of MCV. This assay readily and reproducibly amplified MCV DNA from the known source

of MCV in fresh, frozen MCC. We demonstrated that this PCR assay could detect MCV in as few as 10 cells. No DNA controls and PBL samples used as negative controls yielded a Ct of 40 as expected. The Ct values for the confirmed MCC FFPE samples ranged from 18 to 40, with a mean value of 31, demonstrating heterogeneity in the association of MCV with MCC. PCR assay was readily able to amplify MCV independent of the age of the MCC tissues. All duplicate MCC tissues showed strong concordance in detection. Level of MCV DNA associated in duplicate biopsies was tightly concordant in six of nine. Diagnostic tissue from one patient included three independent sites, biopsied at different time points in the course of the disease. MCV was detected in two of these biopsies but the third axillary metastatic MCC tissue was negative. An inverse correlation was suggested between patient survival and the level of MCV DNA observed. MCV DNA was not detected in nine colon cancer tissues, but a low level (Ct 38) of MCV DNA was reproducibly detected in three of 20 breast cancers.

## Conclusion

We have developed and validated a PCR assay to reliably detect and quantify MCV DNA from archived FFPE tissues. Using this newly developed real time PCR assay, we confirmed the association of MCV with MCC and demonstrated heterogeneity of MCV association with MCC. Furthermore, this PCR assay suggests that MCV can be detected in DNA from tissues other than MCC and thus might be useful in assessing the prevalence of MCV in various malignancies and body sites. Much remains to be done, including investigation of 1) correlations of MCV positivity in MCC with other biomarkers and 2) the possibility that MCC tissues demonstrate immunohistochemical cross-reactivity with available polyoma T virus reagents, both of which are in progress.

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